ADENO-X RAPID TITER PROTOCOL ADOPTED FROM CLONETECH

This protocol is adapted from BD Bio Sciences-Clonetech Protocol by the Gene Expression Lab.
This protocol is for use with BD Bio Sciences-Clonetech Systems. For additional technical inquiries, contact Technical Service at 800-662-2566 *or www.clonetech.com*

INFECTION OF CELLS
FIXING CELLS/ADD ANTIBIOTICS
QUANTITATION

Infect Cells

1) Seed 1 mL of healthy HEK 293 cells (5x10E4cells/ mL) in each well of a 24 well plate. Use standard growth medium (e.g., DMEM + 5%FBS + antibiotics).
2) Using 2% medium as diluent, prepare 10 fold dilutions of viral samples from 10E2-10E11/ mL.
3) Remove the growth media, ass 500 mL of viral dilution slowly to each well. Mix well.
4) Incubate cells at 37°C in 5% CO2 for 65 hours.
5) Aspirate medium. Allow the cells to dry in hood for 5 minutes .

Fix Cells and Add Antibiotics

1) Fix cells by very gently adding 1 mL of ice cold 100% Methanol to each well.
2) Incubate the plate at -20°C for 10 minutes.
3) Aspirate methanol. Gently rinse wells three times with 1 mL PBS + 1%BSA.
4) Dilute Mouse Anti-Hexon Antibody 1:1000 in PBS + 1%BSA.
5) Aspirate final rinse from wells. Add 0.5 mL of Anti-Hexon Antibody dilution to each well. Incubate at 37°C for 1 hour.
6) Aspirate Anti-Hexon Antibody. Gently rinse the well three times with 1 mL PBS + 1% BSA.
7) Dilute Rat Anti-Mouse Antibody (HRP conjugate) 1:500 in PBS + 1% BSA.
8) Aspirate final rinse from the wells. Then add 0.5 mL of Rat Anti-Mouse Antibody (HRP conjugate), dilution to each well. Incubate at 37°C for 1 hour.
9) Prior to removing the Rat Anti-Mouse Antibody (HPR conjugate), prepare DAB working solution by diluting 10x DAB substrate 1:10 with 1x stable

Peroxidase buffer (you will need 500 µL DAB solution per assay well). Allow the 1x DAB solution to come to room temperature.

Note: Do not allow 10x DAB substrate to come to room temperature. Aspirate Rat Anti-Mouse (HRP conjugate) dilution. Gently rinse each well three times with 1 mL PBS + 1% BSA.

Develop Color and Quantitate

1) After removing final PBS + 1% BSA rinse, ass 500 mL of DAB working solution to each well. Incubate at room temperature for 10 minutes.
2) Aspirate DAB and add 1 mL PBS to each well.
3) Count brown/black positive cells. Calculate the mean number of positive cells in each well.